

Humidity and summer precipitation dynamics recorded in tree rings

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Summary

Motivation and Management implications

Southwestern ecosystems currently experience high interannual variability in precipitation, and this variability is expected to increase under many global change scenarios. Due to the economic importance and carbon storage potential of forests, there is a great deal of interest in determining the response of forest ecosystems to global change factors such as increased atmospheric CO₂, nitrogen deposition, and changes in temperature and precipitation. Like other ecosystems of the southwestern U.S., forest systems are sensitive to moisture availability and are likely to be more sensitive to changes in precipitation than other climate change factors. As a result, understanding physiological and growth responses to precipitation variability is essential to predicting forest responses to future precipitation regimes. Unfortunately, the size and longevity of forest trees impose severe constraints on the scope of monitoring and manipulative studies, limiting our understanding of how mature trees respond to environmental change. By using a combination of stable isotope techniques and traditional dendrochronology, we can establish long-term records of interannual climate variability and the physiological and growth responses of forest trees to climate and increasing atmospheric CO₂ concentration.

Determining water source using stable isotopes

The stable isotope ratios of oxygen (O) and hydrogen (H) in tree-ring cellulose can provide information about the water sources used by trees and the humidity conditions in which they are growing. The ratios of heavy to light isotopes of O (¹⁸O/¹⁶O) and H (D/H) in precipitation vary seasonally, because these ratios are strongly controlled by cloud temperature. Winter precipitation is depleted in the heavy isotopes of O and H, relative to summer precipitation. The different water sources available to trees also vary isotopically; for example, groundwater is often recharged by winter storm events or snowmelt, and will have a winter isotopic signature, while surface soil water will reflect the signature of recent precipitation events, and may have a summer signature. Trees record their water source as they grow because the cellulose laid down in annual growth rings carries the isotopic signature of the water used by the tree during each growing season.

Sampling strategy

Source water. We hope to establish several long chronologies of tree water sources in the Southwest, in order to reconstruct historical variations in the intensity of summer precipitation. The first step in doing this is to quantitatively determine whether trees currently use summer precipitation at several sites, including Bryce Canyon National Park. As experimental controls, we would like to measure the water sources of Ponderosa pine trees that are likely to have access to groundwater (for example, trees growing within a drainage), and trees that are unlikely to have access to groundwater,

such as on well-drained slopes. We will collect 3-4 small twigs from 10 living, mature trees in each topographic position (for a total of 20 trees) at 3 time points during the 2000 growing season, and will measure the isotopic composition of xylem water extracted from the twigs. A tree that uses summer precipitation will have xylem water that is enriched in the heavy isotopes of O and H, relative to a tree that uses only groundwater. At each sampling visit, we will collect 4-6 small soil samples and water samples from existing springs or wells. We will also request access to park weather records, and will ask park personnel to help us by saving precipitation samples from the park weather station for isotope analyses. These data will allow us to calculate the proportion of water obtained from each of the water sources available to the trees.

Chronology development. If we determine that the trees are using available summer precipitation, we would like to establish long-term chronologies of tree water source using measurements of the isotopic composition of tree-ring cellulose. This step will involve taking 4 increment cores (5mm diameter) from each of 5 trees of Ponderosa pine and 5 nearby cottonwood trees, extracting the cellulose from the late wood of each annual increment, and measuring the O and H isotope ratios. Ponderosa pine will provide a record of variation in summer precipitation, as wet summers will be reflected in higher isotope ratios than dry summers. Cottonwood will provide a record of past variation in humidity, as this species has a relatively constant water source and variation in isotope ratios will reflect past humidity conditions. We will also measure growth increments and the carbon isotope ratio of each ring, which will provide information on the physiological response of the trees to past variation in summer precipitation and humidity. Although we would prefer to sample old trees, this step does not require that the entire chronology be done on living trees, so we could sample young trees for recent years and extend the chronology using well-preserved old snags, stumps or logs if necessary.

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Introduction

The response of forests to environmental change is important to both land managers and global change scientists because of their aesthetic and economic value and potential to store carbon. Predicting future forest productivity requires understanding the physiological and growth responses of trees to multiple environmental variables, including precipitation, temperature, nutrient availability, and atmospheric CO₂ concentration. The size and longevity of forest trees limits the scope of manipulative and monitoring studies, limiting our understanding of the responses of mature trees to environmental change. One way to move beyond the limits of short-term studies is to determine tree responses to past environmental variability, using the record of tree growth and physiology provided by annual growth rings. The hydrogen and oxygen isotope ratios of tree ring cellulose can provide information on the water sources used by trees and the humidity conditions experienced at the leaf surface. In addition, the carbon isotope composition of tree-ring cellulose can provide a long-term record of physiological responses to variation in precipitation and humidity, and to increasing atmospheric CO₂. We will analyze the hydrogen and oxygen isotope ratios of tree-ring cellulose from Ponderosa pine growing at several sites on the Arizona monsoon boundary to construct a long-term record of variation in summer precipitation and humidity. We will then use carbon isotope analysis and tree-ring growth chronologies to determine the physiological and growth responses of Ponderosa pine to past precipitation and humidity conditions, and to increasing atmospheric CO₂.

The release of fossil carbon by human activities has changed atmospheric composition dramatically over the past century. Atmospheric CO₂ concentration has increased from ~270 ppm in the middle of the last century to ~360 ppm today, and is continuing to increase at a rate of 1-2 ppm yr⁻¹ (Keeling et al. 1995). The increased concentrations of CO₂ and other trace gases are altering global energy balance, which is likely to lead to climatic change (IPCC 1996). Understanding forest responses to changes in climate and atmospheric composition is important for land management and for predicting biosphere feedbacks to atmospheric composition and regional energy balance. Arid and semi-arid ecosystems are among the most sensitive to change (IPCC 1996), and they respond strongly to patterns of resource availability, particularly the variability and timing of precipitation (Noy Meir 1973, Ehleringer 1998). Productivity in western U.S. forests is controlled primarily by site water balance (Waring 1998), so changes in the amount and variability of precipitation are likely to be the most important component of global change influencing the productivity of southwestern U.S. forest systems.

Ponderosa pine (*Pinus ponderosa*) is the most important pine species in western North America, and it is second only to Douglas fir (*Pseudotsuga menziesii*) in its importance as a timber species in the U.S. (Harlow et al. 1991). Ponderosa pine has a broad geographic range, extending from Mexico into Canada, including throughout the Rocky Mountains and the Intermountain West. Although it attains its maximum growth on relatively moist slopes of the Siskiyou and Sierra Nevada Mountains, it has a high drought tolerance and occurs on dry sites with nut pines and junipers. Due to its importance as a timber species and as a major component of western U.S. forest ecosystems, the response of Ponderosa pine to environmental change is of great interest to land managers and ecologists. Drought tolerance in Ponderosa pine appears to result from drought avoidance, rather

than high water use efficiency, as it has high transpiration rates when water is abundant and closes stomata quickly in response to water stress (Zhang et al. 1997). Growth of Ponderosa pine has been found to respond positively to water availability in manipulative studies (Zhang et al. 1996, Zhang et al. 1997) and in surveys of tree ring growth increments (Cleaveland 1986, Peterson et al. 1993). Such studies suggest that Ponderosa pine will respond strongly to changes in water availability associated with global change.

Precipitation in the Colorado Plateau region of the southwestern U.S. has a bimodal distribution, with peaks in the winter and summer separated by long, relatively dry periods (Hereford & Webb 1992). Total seasonal rainfall is a linear function of the frequency of storm events (Hereford & Webb 1992, Ehleringer 1994). There has been considerable interannual variability in precipitation over the past 80 years, during which climate records have been kept throughout the region (Hereford & Webb 1992, Ehleringer 1994). Some of this variability is related to the El Niño/Southern Oscillation (ENSO) cycle, which appears to affect both winter and summer precipitation. Cayan and Webb (1992) determined that there were more winter precipitation days during El Niño years, relative to La Niña years (26% vs. 16%), which may equate with more frequent storms or storms that last longer. Hereford and Webb (1992) determined that the typical result of El Niño conditions in the Colorado Plateau region is normal to above-normal summer precipitation. El Niño and La Niña years represent the extremes of precipitation variability, but there is also considerable variation among years not influenced by ENSO. This variability has been associated with changes in the latitudinal gradient of sea surface temperatures (SST) off the Pacific Coast of the U.S. (Carleton et al. 1990).

Past variability in the amount and seasonality of precipitation in the southwestern U.S. provides an opportunity to study the physiological and growth responses of trees to the types of climatic change predicted by global change models. Tree-ring sequences have long been used to infer past climate conditions, based on relationships between growth increments and growing conditions. More recently, analysis of the stable isotope ratios of hydrogen, oxygen, and carbon have been used to reconstruct past climates based on correlative relationships between the isotopic composition of tree-ring cellulose and climate factors such as temperature and humidity. By combining stable isotope and standard dendrochronological techniques, it may be possible to characterize past patterns of climate variability and to determine the physiological and growth responses of trees to that variability.

Stable isotope terminology. The stable isotope composition of a material is generally presented relative to that of a standard using the delta (δ) notation in parts per thousand (‰):

$$\delta = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] * 1000 \quad (1)$$

where δ is the isotope ratio (δD , hydrogen isotope ratio; $\delta^{18}O$, oxygen isotope ratio; $\delta^{13}C$, carbon isotope ratio) and R is the molar ratio of the heavier to the lighter isotopes (D/H , $^{18}O/^{16}O$, $^{13}C/^{12}C$). The standard for both hydrogen and oxygen is Standard Mean Ocean Water (SMOW), which has R values of $D/H = 0.00015576$ and $^{18}O/^{16}O = 0.0020052$. The standard for carbon is a Pee Dee Belemnite (PDB) with a value of $^{13}C/^{12}C = 0.0112372$.

Hydrogen and oxygen isotope ratios. Variation in the δD and $\delta^{18}O$ of tree-ring cellulose results from variations in source water isotopic composition (Ehleringer & Dawson 1992) and from isotopic effects of leaf water enrichment, biochemical fractionation, and post-

photosynthetic exchange processes (Yakir 1992). The water sources available to plants differ in their isotopic composition, both temporally and spatially. The isotopic signature of precipitation varies seasonally and with latitude and altitude as a result of temperature effects on fractionation during condensation (Craig 1961, Dansgaard 1964).

Groundwater sources reflect the average isotopic composition of annual precipitation inputs, unless more ancient water persists due to slow turnover (Ehleringer & Dawson 1992). Surface soil water reflects the isotopic composition of recent precipitation events, while deeper soils may retain the signature of previous recharge events such as large storms or snowmelt. Stream water originating from snowmelt will carry the isotopic signature of snow, while runoff from summer rain events will reflect the isotopic composition of summer precipitation. Because there is no fractionation during water uptake by plants, the water in roots and in suberized stems has an isotopic composition that is the weighted average of the current water sources being used by a plant at the time of measurement (White et al. 1985, Dawson & Ehleringer 1991).

Measurements of the isotopic composition of xylem water have been used to determine plant water sources over time, and to determine partitioning of water sources among species in a given community (reviewed by Ehleringer & Dawson 1992, Ehleringer et al. 1993). White et al. (1985) were among the first to use stable isotope techniques to show that plants can switch water sources according to their availability. Immediately after a rain event, the xylem water of eastern white pine (*Pinus strobus*) reflected the δD value of the rain water, but as the surface soil dried, the δD of xylem water approached the δD of groundwater at a wetter site, and that of heartwood water at a drier site. Dawson and Ehleringer (1991) used hydrogen isotope analysis to demonstrate that mature trees growing in or in close proximity to a perennial stream in the Intermountain West did not rely on stream water as might be expected, but instead used groundwater. A number of recent studies have shown that, in arid and semi-arid systems of the western United States, some perennial species do not use summer precipitation, although it may account for nearly half of annual precipitation inputs (Ehleringer et al. 1991, Flanagan et al. 1992, Donovan & Ehleringer 1994).

Based on such studies, it is reasonable to expect that tree rings should provide a long-term, integrative record of plant water sources. However, the isotopic composition of tree-ring cellulose is also influenced by fractionation and exchange processes that occur in the leaf and during photosynthesis and cellulose synthesis (Yakir 1992). Although there is no fractionation during water uptake or transfer through the phloem, isotopic enrichment occurs when water reaches tissues subject to evaporative loss, such as leaves and unsuberized stems. This enrichment is in part a function of the vapor pressure gradient from the leaf to the air (see Methods section; Craig & Gordon 1965, Flanagan et al. 1991a, Flanagan et al. 1991b).

Further fractionation occurs during photosynthesis, where discrimination during electron transport results in a pool of reductant that is depleted in the heavier isotope of hydrogen (Estep & Hoering 1981). Yakir & DeNiro (1990) determined this fractionation factor to be -171‰ by comparing the δD of cellulose formed under autotrophic and heterotrophic conditions. Discrimination against ^{18}O occurs during exchange between carbonyl-bound oxygen and water, resulting sucrose that is ~27‰ enriched relative to leaf water. There is further fractionation against the heavier isotope of hydrogen during heterotrophic carbohydrate metabolism, resulting in cellulose that is ~158‰ enriched relative to the sucrose used in cellulose synthesis (Yakir & Deniro 1990, Luo & Sternberg 1992b). In addition, a proportion of the carbon-bound oxygen and hydrogen is exchanged with oxygen and hydrogen in stem water during cellulose synthesis. Roden and Ehleringer (1999) have determined exchange rates of about 42% for oxygen and about 36% for

hydrogen. Using model equations (discussed in Methods section), it is possible to use measurements of tree-ring cellulose isotope ratios to determine the isotopic composition of source water and the leaf water enrichment that results from humidity conditions.

Carbon isotope composition. While the hydrogen and oxygen isotope compositions of tree-ring cellulose can provide information on environmental conditions experienced by a plant, we can also obtain information about the physiological responses to those conditions from the carbon isotope composition ($\delta^{13}\text{C}$) of tree rings. Ehleringer (1993) has proposed that the ratio of intercellular to ambient CO_2 concentrations (c_i/c_a) is a physiological set point in gas exchange metabolism that can be useful in understanding how plants respond to environmental change. The c_i/c_a ratio is a set point that represents the integration and coordination of the mechanical and biochemical processes that regulate the fluxes of CO_2 and water, remaining relatively stable as the absolute flux rates change in response to an environmental change (Ehleringer et al. 1993). Differences in the c_i/c_a ratio among plants result from differences in stomatal conductance and/or photosynthetic demand for intercellular CO_2 (Farquhar et al. 1989, Ehleringer et al. 1993). The carbon isotope ratio in plant material provides an integrative measure of the c_i/c_a ratio over time, due to the influence of c_i on carbon isotopic discrimination during carbon fixation (Farquhar et al. 1982). The carbon isotopic discrimination (Δ) in C3 plants is related to c_i/c_a as:

$$\Delta = a + (b - a) c_i/c_a \quad (2)$$

where a is the fractionation occurring due to diffusion in air (4.4 ‰), and b is the net fractionation caused by carboxylation (mainly discrimination by RuBP carboxylase, approximately 27 ‰).

Differences in leaf Δ values are often related to stomatal responses to environmental conditions (Comstock & Ehleringer 1992). Environmental factors that might influence stomatal activity include humidity (Comstock & Ehleringer 1993), water stress (Guy et al. 1986), hydraulic constraints (Meinzer et al. 1993), and atmospheric CO_2 concentration (Polley et al. 1992). Any factor that causes a decrease in stomatal conductivity can have a negative effect on photosynthesis and growth by reducing c_i . Such effects on c_i are recorded in the $\delta^{13}\text{C}$ of organic matter, as a 15ppm shift in c_i causes an approximately 1‰ shift in Δ .

Superimposed on the climatic variability of the last 150 years is a gradual increase in atmospheric CO_2 concentration and a decrease in atmospheric $\delta^{13}\text{C}$ due to the release of ^{13}C -depleted fossil carbon into the atmosphere (Friedli et al. 1986, Keeling 1986). This change in atmospheric CO_2 has important implications for interpreting the relationship between tree-ring $\delta^{13}\text{C}$ and climate over time, and provides an opportunity to determine long-term physiological responses to increasing atmospheric CO_2 . At this time, it is unclear how increasing atmospheric CO_2 affects the c_i/c_a of plants over the long term. Polley et al. (1993) exposed plants to the atmospheric CO_2 concentrations experienced during glacial-interglacial times (~150-300 ppm), and found that plants maintained a constant c_i/c_a over this range of ambient CO_2 concentrations. As a result, photosynthesis was reduced at lower CO_2 concentrations and responded positively and linearly to increasing CO_2 (Polley et al. 1992). Similarly, herbaria samples of Mediterranean C3 plants exhibited a constant c_i/c_a at atmospheric CO_2 concentrations ranging from ~280-310 ppm (Peñuelas & Azcón-Bieto 1992). Beerling (1996) also observed maintenance of constant c_i/c_a ratios in two historical sequences of leaves: herbarium samples of the woody dwarf shrub *Salix herbacea* L. spanning the past two centuries, and a 30,000 year sequence of *Pinus flexilis* leaves from the Pleistocene. In both of these studies of historical leaf samples, decreased stomatal density contributed to the maintenance of

constant c_i/c_a ratios as atmospheric CO_2 concentrations increased (Peñuelas & Azcón-Bieto 1992, Beerling 1996).

In contrast, Ehleringer and Cerling (1995) found that c_i/c_a values of preserved leaf litter of *Prosopis alba* from the Atacama Desert have decreased over the last 100 years, during which atmospheric CO_2 has increased ~60 ppm. Leaf nitrogen concentrations also remained constant over this period (Ehleringer et al. 1992), which, combined with the maintenance of a constant c_i , suggests that photosynthetic rates remained constant while water use efficiency increased with increasing atmospheric CO_2 (Ehleringer & Cerling 1995). Similarly, Peñuelas and Azcon-Bieto (1992) found a decrease in $\delta^{13}\text{C}$, and thus c_i/c_a , in herbaria samples representing an atmospheric CO_2 concentration of ~350 ppm, relative to samples representing CO_2 concentrations below 310 ppm. These results suggest that plants tightly regulate c_i/c_a across the range of atmospheric CO_2 concentrations typical of the last several million years of evolutionary change, but have less control over c_i/c_a at CO_2 concentrations outside this range (Ehleringer & Cerling 1995). Regardless of the type of response (maintenance of c_i/c_a or of a constant c_i), increasing atmospheric CO_2 will influence the interpretation of physiological and growth responses to climate variability by independently affecting growth and/or water use efficiency.

Experimental Plan

The cellulose of tree rings from Ponderosa pine and nearby cottonwood trees will be analyzed for δD and $\delta^{18}\text{O}$ in order to establish a record of interannual and seasonal variability in precipitation and humidity at several sites in southern Utah and northern Arizona. Ponderosa pine uses both summer and winter precipitation (variable isotopic composition), while cottonwood relies on stream water derived from snowmelt (relatively fixed isotopic composition). As the differences in isotopic composition between summer and winter precipitation are large relative to humidity effects on leaf water enrichment, the isotopic composition of Ponderosa pine cellulose will reflect the relative importance of summer and winter precipitation. In contrast, since cottonwood uses a water source with a relatively fixed isotopic composition, cellulose δD and $\delta^{18}\text{O}$ will primarily be influenced by humidity effects on leaf water enrichment. Data from the last ~80 years of growth will be tested against local weather records at each site, in order to validate modeled values. Analysis of tree rings from years preceding the initiation of local climate records will produce a record of the frequency and intensity of shifts in the Arizona monsoon during this period. Measurement of $\delta^{13}\text{C}$ and tree growth rates will allow evaluation of the effects of past climatic variability on physiology and growth of these important forest species. In addition, $\delta^{13}\text{C}$ analysis will be used to determine the effects of recent increases in atmospheric CO_2 on tree physiology and growth.

Methods

Sample collection. In order to confirm that Ponderosa pine uses summer precipitation, we will analyze xylem water from trees that are (1) likely to have access to groundwater and (2) unlikely to have groundwater at several sites in southern Utah and northern Arizona. At 3 time points during the 2000 growing season, we will collect 3-4 small twigs from each of 10 trees in 2 topographic positions at each site, and extract the xylem water for isotope analysis. We will also collect 4-6 soil samples for soil water analysis, and will collect precipitation and groundwater from springs or existing wells. Isotope analysis of these water sources will allow us to calculate the proportion of water the trees

are obtaining from each available source. At sites where we are able to confirm that trees are using summer precipitation, we will take increment cores from 5 mature trees of Ponderosa pine and cottonwood for long-term chronologies. Four increment cores will be taken in the cardinal directions and at breast height from each tree. Previous work has shown that these species produce easily distinguishable growth rings.

Tree-ring width chronologies. Growth analyses will be performed on each core in its entirety. Each increment core will be cross-dated with samples from tree-ring chronologies (International Tree Ring Data Bank) established near the study site. Samples will be cross-dated by skeletan plotting (Stokes & Smiley 1968, Fritts 1976). Ring widths from each core will be measured using a sliding-stage micrometer integrated with a computer as an estimate of annual productivity (Robinson & Evans 1980). Each core chronology will be standardized to remove long-term growth trends (Fritts 1976), and the resulting standardized chronologies averaged to characterize the growth rate of individual trees.

Cellulose preparation. At this stage, individual growth rings will be separated from the cores using a razor knife under a dissecting scope. Each ring will then be divided into an "early" and "late" wood portion. The early and late wood portions from the four cores will be pooled, although only late wood will be analyzed for isotopic composition, as organic matter from the previous growing season may be included in early wood. It is important to analyze only the holocellulose of wood because while holocellulose is laid down only during the year the ring is produced, other carbon-based compounds can be deposited in later years. The pooled samples will be ground to 40 mesh and reduced to holocellulose using the method described by Leavitt and Danzer (1993). Each sample is placed in a labeled pouch made of glass fiber filter so as many as 50 samples can be run through the soxlet and bleaching processes at a time. Holocellulose will be isolated from the wood by first extracting resins, waxes, and lipids in a soxlet apparatus using toluene/ethanol and then ethanol solvents. The samples are then boiled in water to remove low molecular weight polysaccharides and then repeatedly bleached with sodium chlorite and glacial acetic acid. At this stage, only holocellulose remains, which is rinsed with deionized water and then dried before stable isotope analyses are conducted.

Hydrogen isotope analysis. The cellulose samples will be nitrated prior to hydrogen analysis. Hydrogen isotope ratios of samples will be determined by an improvement in the zinc-reduction technique described by Coleman et al. (1982). The hydrogen in nitrated cellulose is first converted to water by combustion with cupric oxide and silver foil, under vacuum. After combustion, the hydrogen ends up as water, which is cryogenically moved to a separate reaction tube containing a zinc alloy (regularly produced by J. Hayes' lab, Indiana University). The tube containing the water and zinc alloy is evacuated and baked at 500°C, converting the hydrogen to its diatomic form. The precision of this method is $\pm 1\%$. The resulting purified H_2 is analyzed for δD on an isotope ratio mass spectrometer (Finnigan MAT 252; San Jose, CA).

Oxygen isotope analysis. Oxygen isotope ratios of the holocellulose samples will be determined using the on-line pyrolysis method developed by Werner et al. (1996) and described by Saurer et al. (1998). The isolated holocellulose from each sample is combusted in an elemental analyzer linked to an isotope ratio mass spectrometer (Delta-S, Finnigan MAT, San Jose, CA). Following combustion, the oxygen in the sample is mainly found in the form of CO, with a small percentage as CO₂ (~5%). The sample gases are then carried through a water trap (magnesium perchlorate), a CO₂ trap, and a separation column before entering the mass spectrometer. The removal of CO₂ prevents the formation of CO⁺ ions from dissociation of CO₂ in the ion source, and does not

compromise the $\delta^{18}\text{O}$ measurements. Sauerer et al. (1998) report a precision of $\sim 0.2\%$ for this method.

Carbon isotope analysis. Carbon isotope ratios will be determined by combusting the isolated holocellulose from each sample on an elemental analyzer attached to a delta S isotope ratio mass spectrometer (Ehleringer 1991). Overall precision of an individual measurement of $\delta^{13}\text{C}$ is $\pm 0.11\%$, which is approximately equivalent to a 1.5 ppm change in c_i , or a 0.0047 change in the c_i/c_a ratio.

Model calculations

The isotope ratios of tree-ring cellulose ($\delta\text{D}_{\text{cx}}$, $\delta^{18}\text{O}_{\text{cx}}$) are related to the isotopic compositions of source water ($\delta\text{D}_{\text{wx}}$, $\delta^{18}\text{O}_{\text{ws}}$) and sucrose exported from the leaf ($\delta\text{D}_{\text{sp}}$, $\delta^{18}\text{O}_{\text{sp}}$) by the following model equations (Roden, Lin, & Ehleringer, unpublished):

$$\delta\text{D}_{\text{cx}} = f_{\text{H}} \cdot (\delta\text{D}_{\text{wx}} + \epsilon_{\text{HH}}) + (1 - f_{\text{H}}) \cdot (\delta\text{D}_{\text{sp}}) \quad (3)$$

$$\text{and } \delta^{18}\text{O}_{\text{cx}} = f_{\text{O}} \cdot (\delta^{18}\text{O}_{\text{ws}} + \epsilon_{\text{O}}) + (1 - f_{\text{O}}) \cdot (\delta^{18}\text{O}_{\text{sp}}) \quad (4)$$

where f_{H} and f_{O} represent the fraction of carbon-bound hydrogen and oxygen that exchange with water during cellulose synthesis (0.35 and 0.36 for hydrogen and oxygen, respectively) (Roden & Ehleringer, unpublished results), and ϵ_{HH} and ϵ_{O} are the fractionation factors associated with heterotrophic carbohydrate metabolism for hydrogen (+158‰) and oxygen (+25‰), respectively (Yakir & Deniro 1990, Luo & Sternberg 1992b).

The isotope ratios of sucrose transported in the phloem ($\delta\text{D}_{\text{sp}}$, $\delta^{18}\text{O}_{\text{sp}}$) are related to the isotopic composition of leaf water ($\delta\text{D}_{\text{wl}}$, $\delta^{18}\text{O}_{\text{wl}}$) by the following model equations (Roden, Lin, & Ehleringer, unpublished):

$$\delta\text{D}_{\text{sp}} = \delta\text{D}_{\text{wl}} + \epsilon_{\text{HA}} \quad (5)$$

$$\text{and } \delta^{18}\text{O}_{\text{sp}} = \delta^{18}\text{O}_{\text{wl}} + \epsilon_{\text{O}} \quad (6)$$

where ϵ_{HA} and ϵ_{O} are the fractionation factors associated with photosynthesis (-171‰, and +27‰, respectively) (Yakir & Deniro 1990, Luo & Sternberg 1992b). Thus, sucrose transported from the phloem contains information about humidity conditions, as it carries the signal of leaf water enrichment (Roden & Ehleringer, unpublished).

The evaporative enrichment of leaf water has been described with a general model developed by Craig and Gordon (1965) and later expanded by Flanagan & Ehleringer (1991) and Flanagan et al. (1991):

$$R_l = \alpha^* \left[\alpha_k R_x \left(\frac{e_i - e_s}{e_i} \right) + \alpha_{kb} R_x \left(\frac{e_i - e_a}{e_i} \right) + R_a \left(\frac{e_a}{e_i} \right) \right] \quad (7)$$

Where R is the molar ratio of the heavy to light isotope (subscripts a, l, and x refer to atmospheric water vapor, leaf water, and stem xylem water, respectively); α^* is the

liquid-vapor equilibrium fractionation factor; α_k is the kinetic fractionation factor associated with diffusion in air; α_{kb} is the kinetic fractionation factor associated with diffusion in the boundary layer, ϵ is the partial pressure of water vapor (subscripts i, s, and a refer to intercellular air spaces, leaf surface, and ambient air, respectively). This equation is presented in its general form and values for R and α variables differ for oxygen and hydrogen isotopes. This model is based on the dynamics of a well-mixed, isotopically homogeneous evaporating surface, but not all water in the leaf is subject to evaporative enrichment, resulting in isotopic heterogeneity within the leaf (Yakir et al. 1989, Luo & Sternberg 1992a, Yakir et al. 1994a). To account for the fraction of water in the leaf that is not exposed to evaporation, the isotope composition is calculated as (Flanagan 1993):

$$R_{wl} = R_{modeled} \cdot f_l + R_{ws} \cdot (1-f_l) \quad (8)$$

where R is the molar ratio of the heavy to light isotope (the subscripts wl and modeled refer to leaf water and the value obtained from equation 1, respectively) and f_l is the proportion of bulk leaf water subjected to evaporative enrichment.

Model Constraints. Some important constraints to the models described above must be considered before applying them to climate reconstruction based on the isotopic composition of tree ring cellulose. First, equations 2 and 3, which describe the relationship between the isotopic composition of tree ring cellulose (δ_{cx}), source water (δ_{ws}), and the sucrose used in cellulose synthesis (δ_{sp}) involve two unknown variables. In order to solve for one of the unknown variables (δ_{ws} or δ_{sp}), the other must be known. However, humidity varies by only ~10%, so in systems with large isotopic differences in potential source waters, such as the bimodal precipitation regime of the southwestern U.S., the source water signal will be much stronger than humidity effects on δ_{cx} , allowing approximation of precipitation pattern. To improve these approximations, we can use estimates of the humidity signal in tree-ring cellulose from cottonwood, which uses a water source with a known and constant isotopic composition, to correct for humidity effects on the isotopic composition of Ponderosa pine cellulose.

The second major constraint is in applying the leaf water enrichment model (equations 6 and 7) to humidity reconstructions. While the leaf water enrichment model can be used to make reliable predictions about the isotopic composition of bulk leaf water (Flanagan et al. 1991a, Flanagan et al. 1991b, Flanagan & Ehleringer 1991, Flanagan 1993) the isotopic composition of the metabolic water in the chloroplast may differ significantly from the isotopic composition of the bulk leaf water (Yakir et al. 1994b). In addition, the model requires knowledge of the historical isotopic composition of atmospheric water vapor, which cannot be assumed to be in equilibrium with groundwater in arid environments (Gat 1980). As a result, precise calculations of past humidity conditions cannot be made accurately from established models. However, estimates of δ_{wl} obtained from the models can be used for a relative comparison of humidity conditions.

Model application. Hydrogen and oxygen isotope ratios of tree-ring cellulose from Ponderosa pine will be used to estimate the isotopic composition of each tree's source water for each of the past 160 years. Initial source water estimates will be derived from equations 3-6, assuming that leaf water enrichment is negligible. These estimates will be adjusted to account for humidity effects on leaf water isotopic composition based on the average leaf water enrichment observed in cottonwood for each corresponding year (see below). Water derived from winter precipitation has the isotopic composition of snow

($\delta D_{\text{snow}} \approx -120\text{‰}$; $\delta^{18}O_{\text{snow}} \approx -14\text{‰}$), while water derived from summer precipitation is isotopically heavier ($\delta D_{\text{summer}} \approx -30\text{‰}$; $\delta^{18}O_{\text{summer}} \approx -3\text{‰}$). The intensity of summer precipitation will be apparent in the degree to which δD_{ws} and $\delta^{18}O_{\text{ws}}$ are enriched over δD and $\delta^{18}O$ values typical of winter precipitation. For each year, the intensity of summer precipitation will be estimated as the difference between the calculated values (δD_{ws} , $\delta^{18}O_{\text{ws}}$) and typical winter precipitation values (δD_{snow} , $\delta^{18}O_{\text{snow}}$), averaged among trees.

The δD and $\delta^{18}O$ of tree-ring cellulose from cottonwood will be used to estimate humidity conditions for each of the past 160 years. Humidity conditions will be estimated based on the degree of leaf water enrichment determined using equations 3-6, assuming δD_{ws} and $\delta^{18}O_{\text{ws}}$ have been constant. Values for δD_{ws} and $\delta^{18}O_{\text{ws}}$ will be obtained by measuring the isotopic composition of cottonwood xylem water during the growing season preceding tree-ring sample collection. Leaf water enrichment in Ponderosa pine will be approximated from the degree of leaf water enrichment (relative to source water) determined for cottonwood. Precise values for atmospheric humidity (or the vapor pressure gradient across the leaf surface) cannot be determined, as many of the conditions required for application of the leaf water enrichment model (equations 7-8) are unknown. However, the relative degree of leaf water enrichment will provide a context for comparing humidity conditions across years. For each year, humidity will be estimated as the difference between calculated leaf water values (δD_{wl} , $\delta^{18}O_{\text{wl}}$) and water source values (δD_{ws} , $\delta^{18}O_{\text{ws}}$), averaged among trees.

The $\delta^{13}C$ of tree-ring cellulose from Ponderosa pine will be used to determine the c_i/c_a ratio for each growth ring. This ratio is a set point that represents the integration and coordination of the mechanical and biochemical processes that regulate the fluxes of CO_2 and water. The c_i/c_a ratio will be calculated from equation 2, using Δ calculated from the $\delta^{13}C$ value of tree-ring cellulose and atmospheric $\delta^{13}C$ from the literature.

Records of summer precipitation intensity and humidity will be constructed using the values determined as described above. It is important to note that these records will not provide absolute values for summer rainfall and humidity, but will characterize the relative magnitude and direction of deviations from mean conditions. The constructed records will be compared to local historical records, which date back to ~1917. The accuracy of the constructed records will be tested by regressing estimated values against historical values. To determine whether c_i/c_a and growth have responded to past variation in summer precipitation and humidity, the constructed records will be regressed against values of c_i/c_a , as determined from $\delta^{13}C$, and tree-growth increment. It is expected that c_i/c_a and growth will be relatively lower during years with low summer precipitation and/or low humidity.

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